View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

European Journal of Pharmacology 690 (2012) 170–175

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

The 2-nitrate-1,3-dibuthoxypropan, a new nitric oxide donor, induces vasorelaxation in mesenteric arteries of the rat ☆

Maria S. França-Silva [a], Melissa N. Luciano [a], Thaís P. Ribeiro [a], Juliane S.F. Silva [a], Alexsandro F. Santos [b], Karime C. França [c], Lia S. Nakao [c], Petrônio F. Athayde-Filho [b], Valdir A. Braga [a,*,1], Isac A. Medeiros [a,1]

[a] Biotechnology Center, Federal University of Paraíba, P.O. Box 5009, 58.051-970 João Pessoa, PB, Brazil
[b] Department of Chemistry, Federal University of Paraíba, João Pessoa, PB, Brazil
[c] Department of Pathology, Federal University of Paraná, Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 5 April 2012
Received in revised form
19 June 2012
Accepted 20 June 2012
Available online 14 July 2012

Keywords:
Organic nitrate
Nitric oxide
Vasorelaxation

ABSTRACT

The reduced availability of nitric oxide (NO) is associated with cardiovascular diseases. Therefore, NO donors such as organic nitrates are useful for the treatment of these disorders. The 2-nitrate-1,3-dibuthoxypropan (NDBP) is an organic nitrate synthesized from glycerin, which the pharmacological effects have not been investigated. In this study we evaluated the vasorelaxant effect induced by NDBP in superior mesenteric artery from rats. In phenylephrine pre-contracted artery rings, NDBP (10^{-8}–10^{-4} M) elicited concentration-dependent and endothelium-independent relaxation, which were attenuated by hydroxocobalamin-HDX (30 μM), a NO extracellular scavenger, and 1-H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one-ODQ (10 μM), an inhibitor of soluble guanylyl cyclase (sGC). In addition, the NDBP-induced relaxation was reduced by non-selective K^+ channels blocker KCl (20 mM) or selective K^+ channels blockers such as tetraethylammonium-TEA (B_{KCa}, 1 mM), charybdotoxin-ChTX (B_{KCa}, 100 nM), glibenclamide (K_{ATP}, 1 μM) and 4-aminopyridine-4-AP (K_V, 1 mM). In preparations with ODQ (10 μM) plus TEA (1 mM), the response was virtually abolished. In rat smooth muscle cells culture, NDBP (10^{-6}–10^{-4} M) caused concentration-dependent increases in NO levels. These findings suggest that NDBP causes vasorelaxation through NO generation and activation of the sCG/cGMP/PKG pathway.

© 2012 Elsevier B.V. Open access under the Elsevier OA license.

1. Introduction

The nitric oxide (NO) is an important molecule in the modulation of cardiovascular function (Shapoval, 2004). One of its actions consists of vascular tone regulation by direct vascular smooth muscle cells relaxation via activation of soluble guanylyl cyclase (Schmitt and Dirsch, 2009). The vascular tone in turn, is one of the major determinants of blood flow resistance, playing important role in blood pressure regulation (Jackson, 2000). Therefore, dysregulation of the vascular tone contributes to the generation and maintenance of some cardiovascular diseases, which have been linked to decreased NO availability (Nassem, 2005).

The mechanisms underlying the vasorelaxation elicited by NO involve activation of guanylate cyclase (sGC), increasing intracellular levels of cyclic guanosine monophosphate (cGMP) (Archer et al., 1994), resulting in activation of protein kinase G (PKG), which is an enzyme responsible for the phosphorylation of several proteins involved on vasorelaxation (McDonald and Murad, 1996). Among the targets for PKG, the vascular smooth muscle K^+ channels significantly contribute to vascular tone regulation and their opening results in cell hyperpolarization or repolarization due to K^+ efflux, followed by subsequent closing of voltage-dependent Ca^{2+} channels, decreasing the intracellular level of Ca^{2+} leading to vasodilatation (Archer et al., 1994; Gurney, 1994; Haddy et al., 2006; Lovren and Triggle, 1998; Sand et al., 2006). Nevertheless, several studies documented that NO may act by a cGMP-independent mechanism, through direct activation of K^+ channels also resulting in vascular relaxation (Bolotina et al. 1994; Mistry and Garland, 1998; Plane et al., 1996).

Due to the role of NO in vascular tone regulation, a decrease in its bioavailability is associated with several cardiovascular diseases (Nassem, 2005), such as hypertension and coronary disease. In this context, potential NO donors, that mimic the role of endogenous NO when applied to biological systems, have presented beneficial effects on these disorders. The organic nitrovasodilators, such as glyceril trinitrate and amyl nitrite, currently used in clinical practice, induce vascular smooth muscle cells relaxation, improving blood supply and thereby presenting a beneficial effect against angina pectoris, pulmonary hypertension,

☆This work has been funded by "Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq" grant number 304718/2011-4.

* Corresponding author. Tel./fax: +55 83 32167511.
E-mail address: valdir@cbiotec.ufpb.br (V.A. Braga).
[1] These authors are joint last authors.

0014-2999 © 2012 Elsevier B.V. Open access under the Elsevier OA license.
http://dx.doi.org/10.1016/j.ejphar.2012.06.043

cardiac ischemia, thrombus formation, among other disorders due likely to NO release (Goyal et al., 2006; Yurtseven et al., 2003). Considering the importance of these compounds in treating cardiovascular diseases, the efforts employed to the discovery and synthesis of novel NO donors, including organic nitrates, from different sources is justified.

Therefore, the aim of the present study was to evaluate the vasorelaxant effect of a glycerin-derived newly synthesized organic nitrate, the 2-nitrate-1,3-dibuthoxypropan (NDBP) (Fig. 1), on vascular smooth muscle cells of rats and to unravel the underlying mechanisms involved in its vasorelaxant effect.

2. Material and methods

2.1. Synthesis of the 2-nitrate-1,3-dibuthoxypropan (NDBP)

According to Santos (2009), NDBP was synthesized at the Department of Chemistry at the Federal University of Paraiba. Its synthesis was divided in three steps: 1. *Obtaining haloydrin*: *haloydrin*(1,3-dichlorine-propan-2-ol) was obtained by reaction between glycerin and hydrochloric acid (HCl) dry in a reaction with 70% yield (Fig. 2A). 2. *Obtaining* 1,3-*diether-propan-2-ol:haloydrin* was added dropwise into a solution of sodium alkoxide, this obtained by reaction between metallic sodium and anhydrous alcohol. As a product we obtained 1,3-diether-propan-2-ol and sodium chloride, according to Fig. 2B. and 3. *Obtaining of* 2-*nitrate*-1,3-*dibuthoxypropan*: by nitration of 1,3-diether-propan-2-ol was originated a compound called 2-nitrate-1,3-dibuthoxypropan (NDBP) (Fig. 2C).

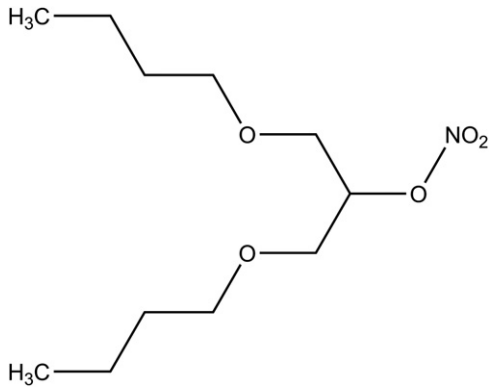

Fig. 1. Structural formula of NDBP.

2.2. Preparation of the NDBP

The NDBP was dissolved in a mixture of distilled water and chremophor and diluted to the desired concentrations with distilled water. In functional studies, the final concentration of cremophor never exceeded 0.01% in the bath and had no effect when tested in control preparations (data not shown).

2.3. Animals

Male Wistar rats (250–300 g), housed under controlled conditions of temperature (21 ± 1 °C) and lighting cycle (lights on: 06:00–18:00 h) with water and food (Labina®, PURINA, Brazil) *ad libitum*, were used in all experiments. Experiments were conducted in accordance with the Institutional Animal Care and Use Committee of the Biotechnology Center–Federal University of Paraíba (CEPA 0310/09).

2.4. Drugs

Phenylephrine chloride, acetylcholine chloride, 1-H-[1,2,4] oxadiazolo–[4,3-a] quinoxalin-1-one (ODQ), tetraethylammonium-chloride (TEA), charybdotoxin (ChTX), 4-aminopyridine (4-AP) and glibenclamide were obtained from Sigma-Aldrich (São Paulo, SP, Brazil). Hydroxocobalamin (HDX) was obtained from Bristol-Myers Squibb (São Paulo, SP, Brasil), and Diaminofluorescein diacetate (DAF-2 DA) from Calbiochem® (São Paulo, SP, Brasil). ODQ and glibenclamide were dissolved in DMSO. All other compounds were dissolved in distilled water.

The composition of the Tyrode's solution used was (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.0; and glucose, 5.6. In depolarizing Tyrode's solution with KCl (20 mM), the concentration of Na^+ was isosmotically adjusted.

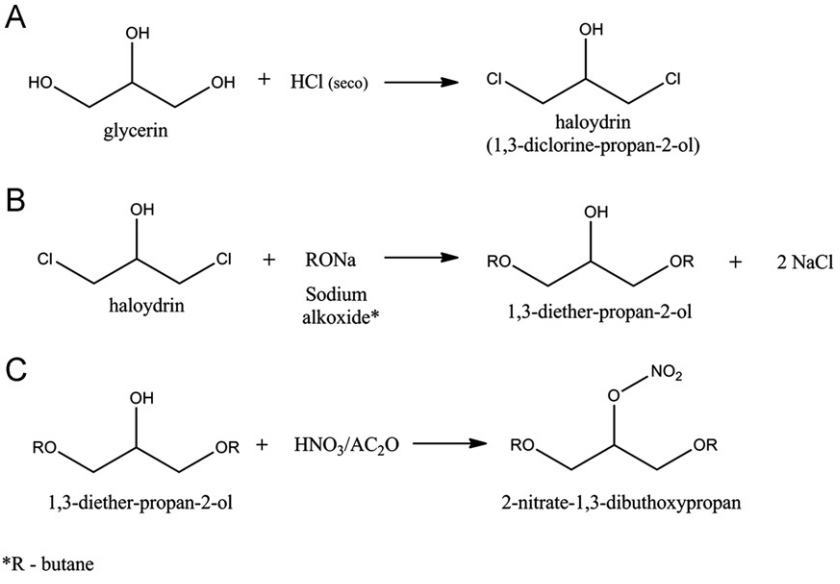

Fig. 2. Synthetic route of 2-nitrate-1,3-dibuthoxypropan (NDBP).

2.5. Evaluation of the vasorelaxant effect induced by NDPB in vitro

After euthanasia, the superior mesenteric artery was isolated, placed in Tyrode's solution and dissected in order to make it free of adhering tissue. In endothelium-denuded experiments, endothelium was removed by rubbing the intimal surface of the vessels. Rings with 1–2 mm were obtained and placed in physiological Tyrode's solution, maintained to 37 °C, gassed with carbogenic mixture (95% O_2 and 5% CO_2) and kept at pH 7.4. All preparations were stabilized under a resting tension of 0.75 g for 1 h. The solution was replaced every 15 min in order to prevent the accumulation of metabolites. The force of contraction was isometrically recorded by a force transducer (Miobath-4, WPI, Sarasota, FL, USA) coupled to an amplifier-recorder (Miobath-4, WPI, Sarasota, FL, USA) and to a computer equipped with an analog-to-digital converter board as described earlier.

The presence of functional endothelium was assessed by the ability of acetylcholine (10 μM) to induce more than 80% relaxation of vessels pre-contracted with phenylephrine (10 μM). Less than 10% of relaxation to acetylcholine was taken as evidence that the vessel segments were functionally denuded of endothelium. The preparations were exposed to: (a) hydroxycobalamin (30 μM), a NO scavenger (Kruszyna et al., 1998); (b) ODQ (10 μM), a soluble guanylyl cyclase inhibitor (Garthwaite et al., 1995); (c) KCl (20 mM), a modulator of potassium efflux (Campbell et al., 1996); and (d) selective blockers for large condutance calcium-sensitive potassium channel (TEA, 1 mM and ChTX, 100 nM), ATP-sensitive potassium channel (glibenclamide, 1 μM), and voltage-operated potassium channel (4-AP, 1 mM) (Adaramoye and Medeiros, 2009; Berg, 2002; Huang and Kwok, 1997; Mombouli and Vanhoutte, 1997). These inhibitors were added 30 min before the application of phenylephrine. In the tonic phase of the contraction, NDBP (10^{-8}–10^{-4} M) was cumulatively added to preparations until a maximum response to the drug accumulation was observed as indicated by a plateau response (approximately 3–5 min). Inhibition was calculated by comparing the response elicited by NDBP in the absence and presence of inhibitors or antagonists in the preparation.

2.6. Measurements of intracellular NO production in aortic rat smooth muscle cells culture

Rat vascular smooth muscular cells (VSMC) line were grown in 24-well plates on the F12 medium and supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin) as previous described (Kojima et al., 1998). Samples were then washed twice with phosphate-buffered saline containing bovine serum albumin and analyzed with 10.000 cells per sample by flow cytometry using the FACSCalibur equipment (Becton Dickinson, San Jose, CA, USA). Bioavailability of NO in VSMC line was quantified by exposing the biological samples to NDBP (10^{-6}, 3×10^{-5} M, 10^{-4} M) or to glyceril trinitrate (GTN), the positive control, for 30 min after pre-incubation with DAF-2 DA (10 mM) for 5 min at 37 °C. The DAF-2 DA is a fluorescent indicator that enables the direct detection of NO under physiological conditions by flow cytometry (Navarro-Antolin and Lamas, 2001). We have detected the concentration of NO release without stimulation (basal fluorescence) and changes in intracellular NO concentration after treatments with NDBP or GTN were expressed as ratio between fluorescence after treatments (F) and basal fluorescence (F_0): F/F_0. Treatments were performed in biological triplicate. Values were expressed as mean ± S.E.M. and analyzed by a WinMDI software version 2.9.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. The maximum effect was considered as the maximum response to phenylephrine (10 μM) for the highest concentration used. Statistical analyses were performed by Student t-test, two-way ANOVA followed by Bonferroni's post-test, or one-way ANOVA with Tukey's post-test when appropriate, using a GraphPad Prism© software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). Significance was considered when $P<0.05$.

3. Results

3.1. Effect induced by NDBP in arteries pre-contracted with phenylephrine

Administration of glycerin-derived organic nitrate, NDBP (10^{-8}–10^{-4} M), produced concentration-dependent vasorelaxation in phenylephrine pre-contracted superior mesenteric artery rings isolated from rats in the presence of functional endothelium ($pD_2=5.85\pm0.10$; $ME=89.5\pm3.4\%$, $n=6$). In addition, removal of endothelium did not alter the response induced by the compound ($pD_2=5.99\pm0.06$; $ME=105.4\pm2.7\%$, $n=6$) (Fig. 3).

3.2. Effect of the NO scavenger and soluble guanylyl-cyclase inhibitor on the relaxation induced by NDBP

In endothelium denuded mesenteric rings pre-treated with hydroxocobalamin (30 μM), NDBP-induced relaxation was significantly attenuated with reduction of pD_2 values, from 5.99 ± 0.06–5.39 ± 0.12, $P<0.05$, $n=6$. The maximum effect (ME) also

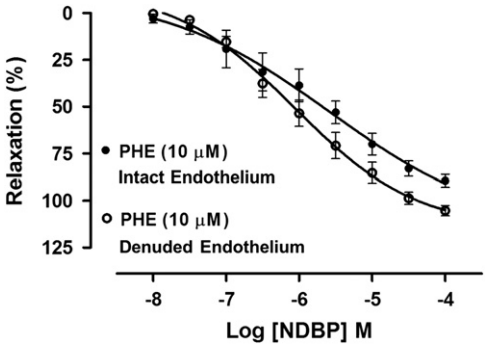

Fig. 3. Concentration-response curves of NDBP (10^{-8}–10^{-4}) in rat mesenteric artery rings ($n=6$). The vasorelaxant effect is expressed as a percentage of relaxation in phenylephrine-induced contraction. Intact endothelium (●), Denuded endothelium (○).

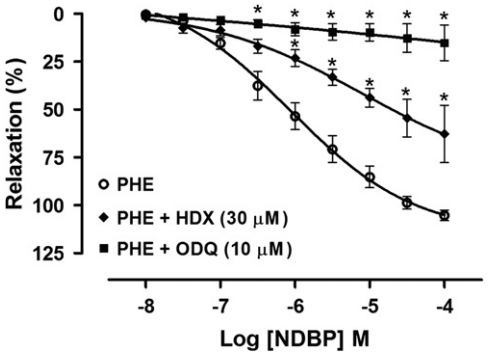

Fig. 4. Effect of hydroxocobalamin (30 μM) ($n=6$) (♦) and ODQ (10 μM) ($n=7$) (■) on NDBP-induced relaxation in phenylephrine-contracted mesenteric artery rings ($n=6$). Values are means ± S.E.M. *$P<0.05$ using two way ANOVA followed by Bonferroni's post test.

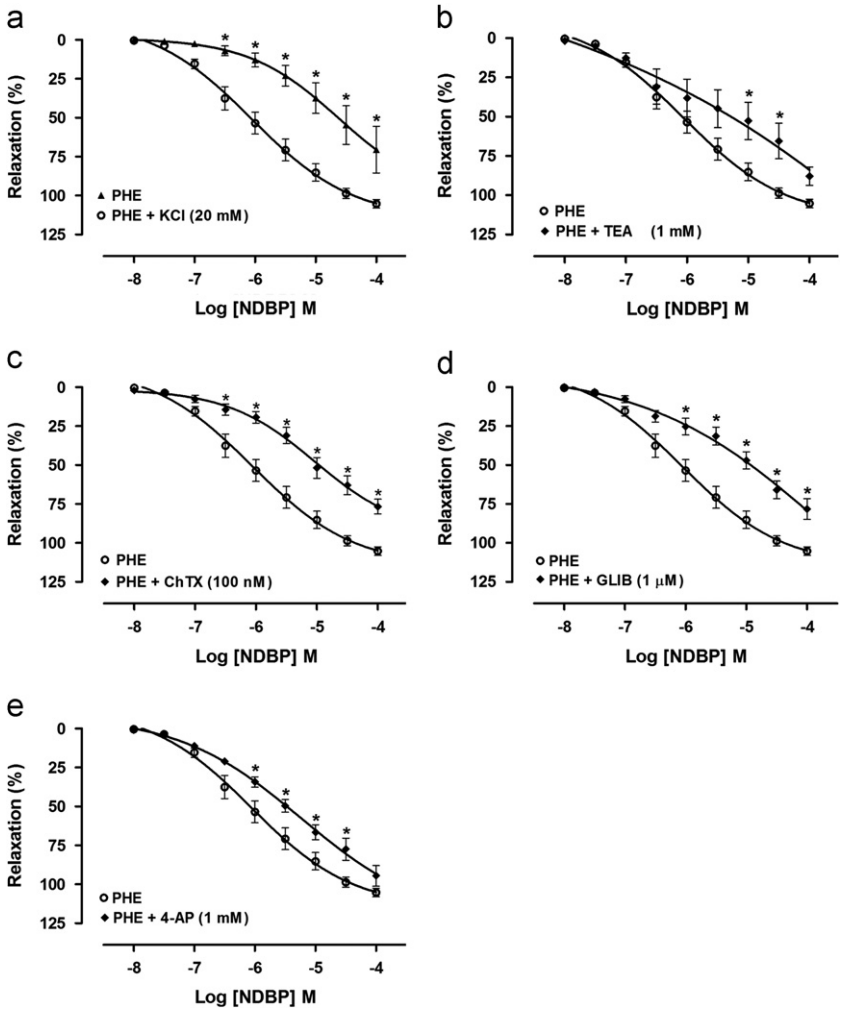

Fig. 5. A. Effect of KCl 20 mM (♦) on NDBP-induced relaxation in phenylephrine-contracted mesenteric artery rings ($n=9$); Effects of TEA 1 mM (♦) in B; ChTX 100 nM (♦) in C; 4-AP 1 mM (♦) in D, and glibenclamide 1 μM (♦) in E on NDBP-induced relaxation in phenylephrine-contracted mesenteric artery rings ($n=6$). Values are means ± S.E.M. $^*P<0.05$ using two way ANOVA followed by Bonferroni's post test.

was reduced (from 105.4 ± 2.7%–62.8 ± 14.9%, $P<0.05$, $n=6$). In addition, in the presence of ODQ (10 μM), the response evoked for NDBP was almost abolished (*ME*: from 105.4 ± 2.7% to 15.2 ± 9.2%, $P<0.05$) (Fig. 4).

3.3. Effect of K^+ channels blockers and the combination of ODQ and K^+ channel blocker on the relaxation induced by the NDBP

Pretreatment with KCl (20 mM), a K^+ efflux modulator, significantly attenuated the vasorelaxant response of NDBP. As a result, KCl (20 mM) shifted to the right the concentration-response curve (pD_2: 5.14 ± 0.13, $n=9$, $P<0.05$) and reduced the maximum effect to 70.6 ± 15.1% (Fig. 5A).

Last but not the least, the vasorelaxant responses induced by increasing concentrations of NDBP (10^{-8}–10^{-4} M) were also significantly rightward shifted, when incubated with TEA (B_{KCa} blocker, 1 mM) ($pD_2=5.58\pm0.16$, $p=0.045$, $n=6$) and 4-aminopyridine (K_V blocker, 1 mM) ($pD_2=5.59\pm0.05$, $p=0.48$, $n=6$) (Fig. 5B e 5E). The ChTX (B_{KCa} blocker, 100 nM) was able to attenuate the NDBP-induced vasodilatation reducing the pD_2 values to 5.35 ± 0.07, $P<0.05$, $n=6$ and maximum effect to 76.76 ± 4.67%, $P<0.05$, $n=6$, as showed in Fig. 5C. The same was found in the presence of glibenclamide (K_{ATP} blocker, 1 μM) ($pD_2=5.43\pm0.08$, $P<0.05$, $n=6$; ME=78.28 ± 4.67%, $P<0.05$, $n=6$) (Fig. 5D). Moreover, the combination of TEA (1 mM) and ODQ (10 μM), produced the same effect as when ODQ was administered alone, eliciting the maximum effect of 6.3 ± 3.6% and 15.2 ± 9.2%, respectively, $n=6$ (Fig. 6).

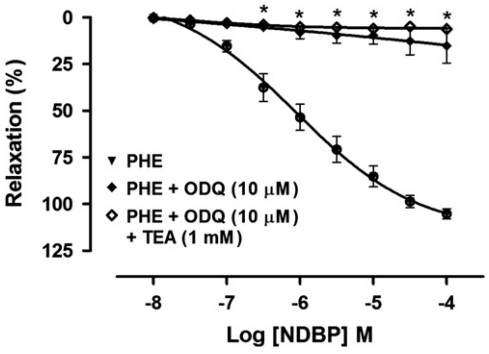

Fig. 6. Effect of ODQ (♦) (10 μM) ($n=7$) and ODQ (10 μM) plus TEA (1 mM) ($n=6$) (◇) on NDBP-induced relaxation in phenylephrine-contracted mesenteric artery rings ($n=6$). Values are means ± S.E.M. $^*P<0.05$ using two way ANOVA followed by Bonferroni's post test.

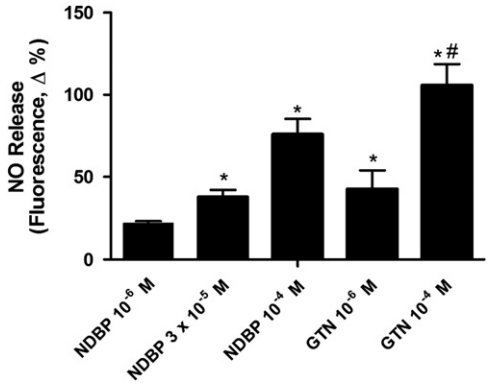

Fig. 7. NO generation in vascular smooth muscle cells (10^6/ml) estimated using Diaminofluorescein diacetate (DAF-2DA). Bar graph showing the effects of glyceryl trinitrate (GTN, $10^{-6}-10^{-4}$ M) as positive control and NDBP (10^{-6}–10^{-4} M) on NO levels expressed as relative fluorescence in aortic rat vascular smooth muscle cells. These data are representative of 3 separate experiments in triplicate, the production baseline was zeroed and the difference was plotted as percentage of fluorescence in different treatments. $P<0.05$ * *versus* basal fluorescence; # *versus* NDBP (10^{-6} M) and NDBP (3×10^{-5} M). Values are shown as mean ± S.E.M.

3.4. Measurements of intracellular NO production after treatment with NDBP

Based on our observations in isolated vessel preparations, we quantified the bioavailability of NO using flow cytometry analysis by quantifying the relative fluorescent using DAF2-DA, which increases in proportion to the amount of NO released in the vascular smooth muscle cells. Therefore, in vascular smooth muscle cells line, NDBP (10^{-6} M, 3×10^{-5} M and 10^{-4} M) was able to increase the relative fluorescence (22.0 ± 1.2; 37.9 ± 4.0; 75.8 ± 9.5; Δ% fluorescence, $n=3$), suggesting the production of NO in the culture medium compared to its control (Fig. 7), corroborating the results obtained in functional studies. There was no statistical difference between NO production of NDBP and to that elucidated by GTN (NDBP, 10^{-6} M: $22.0\pm1,2$ *versus* GTN, 10^{-6} M: 42.5 ± 11.3; and NDBP, 10^{-4} M: 75.8 ± 9.5 *versus* GTN, 10^{-4} M: 105.7 ± 12.9).

4. Discussion

The major finding of this work was that the organic nitrate NDBP, induces vasorelaxation in rat superior mesenteric artery rings through NO release and subsequently activation of the NO-GMPc pathway and K^+ channels activation.

Relaxing substances synthesized and released by endothelial layer are involved in the mechanisms of relaxation in vascular smooth muscle cells. Among those substances is NO, the main vasodilator produced by endothelium. In addition, relaxation can also be induced by exogenous NO, obtained from NO donors. These compounds are able to relax the vessels in a endothelium-independent fashion. In this context, our data pointed out that NDBP relaxed the pre-contracted rat mesenteric rings independent of the endothelium. Based on this observation and supported by the fact that organic nitrates have a history of acting as NO donors, we investigated a possible involvement of the NO-pathway in the mechanisms underlying NDBP-induced vasorelaxation.

Based on our findings showing that hydroxocobalamin (30 μM) attenuated the vasorelaxation of NDBP (Fig. 4), we demonstrated that NO plays a role in the vasodilatation induced by the tested compound. In addition, in the presence of the soluble guanylate cyclase inhibitor, ODQ, the NDBP-induced relaxant response was almost abolished (Fig. 4), further suggesting that the vasorelaxation elicited by NDBP involves NO release and NO/cGMP pathway activation.

It has been widely reported that organics nitrates cause vasorelaxation via activation of the soluble guanylyl cyclase (sGC) by NO, which in turn dose-dependently increases the levels of cGMP, demonstrating that NO is the effector molecule released by the nitrovasodilator, being responsible for causing vasorelaxation. Furthermore, the accumulation of cGMP leads to activation of protein kinases such as PKG. Among the downstream effectors of PKG for vasorelaxation, K^+ channels play an important role in the regulation of smooth muscle contractility and vascular tone, being determinant for the homeostasis of blood pressure. On the other hand, NO can induce vasodilatation by cGMP-independent mechanism throughout direct action on K^+ channels (Bolotina et al., 1994; Mistry and Garland, 1998; Plane et al., 1996).

In order to investigate whether NDBP-induced vasodilatation through the NO release could involve the participation of K^+ channels, we incubated some preparations with KCl (20 mM), which promotes partial blockade of K^+ efflux, reducing the relaxation mediated by the opening of K^+ channels (Gurney, 1994). As a result, increasing the concentration of extracellular K^+ from 4 mM to 20 mM significantly altered the vasorelaxant response induced by NDBP and reduced the maximum effect, suggesting that hyperpolarizing mechanisms mediated by the opening of K^+ channels are part of the effect induced by the compound.

Last but not the least, the vasorelaxant responses induced by increasing concentrations of NDBP (10^{-8}–10^{-4} M) were also significantly rightward shifted, when incubated with tetraethylammonium (TEA, 1 mM), calcium sensitive K^+ channel blocker ($ME=87.9\pm5.8\%$, $P<0.05$, $n=6$), as illustrated in Fig. 4.

The concentration of TEA employed in our studies (1 mM) is known for selectively blocking the large conductance Ca^{2+}-sensitive K^+ channels (Archer et al., 1994). Previous studies indicate that NO and NO donors activate these channels by cGMP-dependent protein kinases, by a direct modulation, or by combining these two mechanisms (Archer et al., 1994; Bolotina et al., 1994). In support to our data, several studies have demonstrated the activation of BK_{Ca} by NO or different NO donors in others vascular tissues, such as pulmonary and cerebral arteries (Bialecki and Stinson-Fisher, 1995; Hempelmann et al., 2001). There is controversy if TEA 1 mM is selective for B_{KCa}, therefore we used charybdotoxin (ChTX, 100 nM), a B_{KCa} blocker, which is more selective than TEA, and the vasorelaxation remained attenuated, confirming the participation of B_{KCa} in the effect induced by NDBP.

In addition to the BK_{Ca} channels, it has been reported that NO hyperpolarizes the membrane through the activation of ATP-sensitive K^+ channels (K_{ATP}) in coronary arteries of humans, causing vasorelaxation (Farouque et al., 2004). In our experiments, after administration of the well known highly selective ATP-sensitive K^+ channels blocker, glibenclamide, the NDBP-induced vasodilatation was significantly attenuated (Fig. 4), suggesting the involvement of K_{ATP} channels in the vasorelaxant effect produced by the compound. Our data are in accordance to results reported by Garland and McPherson (1992). However, Meisheri et al. (1993) reported that glibenclamide did not affect the relaxation mediated by sodium nitroprusside and NOC-7 in rat isolated aorta, suggesting that K_{ATP} channels do not play a role in the vasorelaxation mediated by NO donors in aorta. These divergences could be explained by differences among the NO donors used, methods employed as well as the vascular tissue evaluated.

It has been reported that NO also modulate the activity of voltage-sensitive K^+ channels (Archer et al., 1994). The 4-aminopyridine, a selective blocker of these K^+ channels, significantly rightward shifted the curve concentration-response to NDBP on mesenteric artery rings, confirming the modulation of these K^+ channels by the NO released from NDBP (Fig. 4). The

vasorelaxation elicited by others NO donors such as DEA-NON-Oate also was attenuated by K^+ channel blocker 4-aminopyridine (4-AP) in rat basilar arteries (Sampson et al., 2001).

Among those K^+ channels involved in the response mediated by NDBP, the B_{KCa} and K_{ATP} appeared to be the most important ones, since the blockade with charybdotoxin or glibenclamide, respectively, caused the significant effect in attenuating the vasorelaxant response elicited by the NDBP, being equivalent to the attenuation produced by the non-selective blockade with KCl (20 mM).

It is important to report that the involvement of K^+ channels in the NDBP-induced vasorelaxation seems to be, mainly, dependent of soluble guanylate cyclase activation, since the administration of ODQ virtually abolished the relaxing effect induced by the compound. However, residual vasodilatation found in preparations with ODQ alone was reduced when incubated with ODQ and TEA together, suggesting a direct action of NO in K^+ channels.

Corroborating the functional data, it was observed that the NDBP presents kinetics of NO release in vascular smooth muscle cells similar to glyceryl trinitrate (GTN), an organic nitrate widely known with structure suchlike to NDBP and used in the clinics. Although we have not tested the vasorelaxation effect induced by glycerin trynitrate, glycerin was used as a positive control for measuring NO in vascular smooth muscle cells. A series of elegant studies performed by Miller et al. (2008) aiming to measure NO release during GTN-induced relaxation reported that GNT was able to induce relaxation independent on NO release. However, in that case, authors may have succeed in measuring GTN-derived NO at lower doses because NO was very diluted in the organ bath. In our study, NO was directly measure at cell culture level, been much more sensitive and precise. We believe that, although the potency of NDBP was smaller than that reported for GTN, both NDBP and GTN are able to induce vasodilation via NO production/release. Furthermore, the *in vivo* implications of NDBP organic nitrate as well as its ability to induce tolerance are under investigation.

5. Conclusion

In summary, here we suggest the NDBP as a new NO generator. In support of this concept, under our experimental conditions, NDBP was able to induce relaxation in rat mesenteric rings preconstricted with phenylephrine via NO release and activation of the sGC/cGMP pathway, with participation of K^+ channels. It has also released NO in vascular smooth muscle cell culture. These data seem to be exciting, being the NDBP a possible alternative for cardiovascular diseases treatment; however, several studies regarding the metabolism of the compound, tolerance and physiological significance of the vasorelaxant activity of NDBP awaits further investigation before it can be used in clinical trials.

Acknowledgments

The authors are grateful to José Crispim Duarte and collaborators for technical assistance and Matheus Morais de Oliveira Monteiro for proof reading the final version of the manuscript. This work has been funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq grant number 304718/2011-4.

References

Adaramoye, O.A., Medeiros, I.A., 2009. Endothelium-independent vasodilation induced by kolaviron, a biflavonoid complex from *Garcinia kola* seeds, in rat superior mesenteric arteries. J. Smooth Muscle Res. 45 (1), 39–53.

Archer, S.L., Huang, J.M., Hampl, V., Nelson, D.P., Shultz, P.J., Weir, K., 1994. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. P. Natl. Acad. Sci. USA 91, 7583–7587.

Berg, T., 2002. Analysis of the pressor response to the K^+ channel inhibitior 4-aminopyridine. Eur. J. Pharmacol. 452, 325–337.

Bialecki, R.A., Stinson-Fisher, C., 1995. KCa channel antagonists reduce NO donor-mediated relaxation of vascular and tracheal smooth muscle. Am. J. Physiol. Lung C 268, 152–159.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., Cohen, R.A., 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 368, 850–853.

Campbell, W.B., Gebremedhin, D., Prait, P.F., Herder, D.R., 1996. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ. Res. 78, 415–423.

Farouque, H.M.O., Worthley, S.G., Meredith, I.T., 2004. Effect of ATP-Sensitive Potassium Channel Inhibition on Coronary Metabolic vasodilation in Humans. Arterioscler. Thromb. Vasc. 24, 905–910.

Garland, C.G., McPherson, G.A., 1992. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br. J. Pharmacol. 105, 229–435.

Garthwaite, J., Southam, E., Boulton, C.L., Nielsen, E.B., Schmidt, K., Mayer, B., 1995. Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1-H-[1,2,4]oxadiazo-lo[4,3-a]quinoxalin-1-one. Mol. Pharmacol. 48, 184–188.

Goyal, P., Kiran, U., Chauhan, S., Juneja, R., Choudhary, M., 2006. Efficacy of glyceril trinitrate inhalation in reducing pulmonary arterial hypertension in children with congenital heart disease. Br. J. Anaesth. 97, 208–214.

Gurney, A.M., 1994. Mechanisms of drug-induced vasodilatation. J. Pharm. Pharmacol. 46, 242–251.

Haddy, F.J., Vanhoutte, P.M., Feletou, M., 2006. Role of potassium in regulating blood flow and blood pressure. Am. J. Physiol. Reg. I Physiol. 290, 546–552.

Hempelmann, R.G., Seebeck, J., Ziegler, A., Mehdorn, H.M., 2001. Effects of potassium channel inhibitors on the relaxation induced by the nitric oxide donor diethylamine nitric oxide in isolated human cerebral arteries. J. Neurosurg. 93, 1048–1054.

Huang, Y., Kwok, K.H., 1997. Effects of putative K^+ channel blockers on beta-adrenoceptor-mediated vasorelaxation of rat mesenteric artery. J. Cardiovasc. Pharmacol. 29, 515–519.

Jackson, W.F., 2000. Ion channels and vascular tone. Hypertension 35, 173–178.

Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., Nagano, T., 1998. Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins. Anal. Chem. 70, 2446–2453.

Kruszyna, H., Magyar, J.S., Rochelle, l.G., Russel, M.A., Smith, R.P.E., Wilcox, D.E., 1998. Spectroscopic studies of Nitric Oxide (NO) interations with Cobalamins: reaction of NO with superoxocobalamin(Iii) likely accounts for Cobalamin reversal of the biological effects of NO. J. Pharmacol. Exp. Ther. 285, 665–671.

Lovren, F., Triggle, C.R., 1998. Involvement nitrosothiols, nitric oxide and voltage-gated K^+ channels in photorelaxation of vascular smooth muscle. Eur. J. Pharmacol. 347, 215–221.

McDonald, L.J., Murad, F., 1996. Nitric oxide and cyclic GMP signaling. Proc. Soc. Exp. Biol. Med. 211, 1–6.

Meisheri, K.D., Khan, S.A., Martin, J.L., 1993. Vascular pharmacology of ATP-sensitive K1 channels: interactions between glyburide and K1 channel openers. J. Vasc. Res. 30, 2–12.

Miller, M.R., Grant, S., Wadsworth, R.M., 2008. Selective arterial dilatation by glyceryl trinitrate is not associated with nitric oxide formation *in vitro*. J. Vasc. Res. 45, 375–385.

Mistry, D.I., Garland, C.J., 1998. Nitric oxide (NO)-induced activation of large conductance Ca2-dependent K_ channels (BKCa) in smooth muscle cells isolated from the rat mesenteric artery. Br. J. Pharmacol. 124, 1131–1140.

Mombouli, J., Vanhoutte, P.M., 1997. Endothelium-derived hyperpolarizing actor(s): updating the unknown. Trends Pharmacol. Sci. 18, 252–256.

Nassem, K.M., 2005. The role of nitric oxide in cardiovascular diseases. Mol. Aspects Med. 26, 33–65.

Navarro-Antolin, J., Lamas, S., 2001. Nitrosative stress by cyclosporine A in the endothelium: studies with the NO-sensitive probe diaminofluorescein-2/diacetate using flow cytometry. Nephrol. Dial. Transplant. 16, 6–9.

Plane, F., Hurrell, A., Jeremy, J.Y., Garland, C.J., 1996. Evidence that potassium channels make a major contribution to SIN-1 evoked relaxation of rat isolated mesenteric artery. Br. J. Pharmacol. 119, 1557–1562.

Sampson, L.J., Plane, F., Garland, C.J., 2001. Involvement of cyclic GMP and potassium channels in relaxation evoked by the nitric oxide donor, diethylamineNONOate, in the rat small isolated mesenteric artery. Naunyn Schmiedeberg's Arch. Pharmacol. 364, 220–225.

Sand, A., Andersson, E., Fried, G., 2006. Nitric oxide donors mediate vasodilation in human placental arteries partly through a direct effect on potassium channels. Placenta 27, 181–190.

Santos, A.F., 2009. Novas Perspectivas da Glicerina —Síntese de Novos Nitratos com Propriedades Farmacológicas e Melhoradores de Cetano. Dissertação (Mestrado)-—Universidade Federal da Paraíba, João Pessoa, p. 116.

Schmitt, C.A., Dirsch, V.M., 2009. Modulation of endothelial nitric oxide by plant-derived products. Nitric Oxide: Biol. Chem. 21, 77–91.

Shapoval, L.N., 2004. Nitric oxide: involvement in the nervous control of cardiovascular function. Neurophysiology 36, 466–478.

Yurtseven, N., Karaca, P., Kaplan, M., Ozkul, V., Tuygun, A.K., Aksoy, T., 2003. Effect of glyceril trinitrate inhalation on patients with pulmonary hypertension undergoing mitral valve replacement surgery. Anesthesiology 99, 855–858.